

## ABSTRACT

The present invention relates to a method of determining the genotype of a sample polynucleotide having at least a first variant site. At least a portion of the sample polynucleotide is amplified to obtain first amplicons, the first amplicons including the first variant site. The first amplicons are combined with first and second different polynucleotide controls, the first and second polynucleotide controls differing by at least one base therealong, the position of the at least one differing base corresponding to the first variant site of the sample polynucleotide. A plurality of first duplexes are prepared, each of at least some of the first duplexes comprising (i) a polynucleotide strand of one of the first amplicons and (ii) a complementary polynucleotide strand of the first polynucleotide control. A plurality of second duplexes are prepared, each of at least some of the second duplexes comprising (i) a polynucleotide strand of one of the first amplicons and (ii) a complementary polynucleotide strand of the second polynucleotide control. The first and second duplexes are subjected to temperature gradient electrophoresis (TGE) to obtain first and second electrophoresis data. The genotype of the first variant site of the sample polynucleotide is determined based on the first and second electrophoresis data.